PB85-157394

Zinc, Copper, and Manganese Deficiencies in Prenatal and Neonatal Development, with Special Reference to the Central Nervous System

California Univ., Davis

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Jan 85

U.S. Department of Commerce
National Technical Information Service
NTIS

PB85-157394

EPA/600/D-85/021
January 1985

ZINC, COPPER, AND MANGANESE DEFICIENCIES IN PRENATAL AND NEONATAL DEVELOPMENT, WITH SPECIAL REFERENCE TO THE CENTRAL NERVOUS SYSTEM

John M. Rogers[1], Carl L. Keen,
and Lucile S. Hurley

Department of Nutrition
University of California, Davis
Davis, California 95616

[1]Current Address:
Perinatal Toxicology Branch
Health Effects Research Laboratory

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/D-85/021		PB85 157394/AS

4. TITLE AND SUBTITLE	5. REPORT DATE
Zinc, Copper, and Manganese deficiencies in prenatal and neonatal development, with special reference to the central nervous system	January 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) J. Rogers, C. Keen and L. Hurley	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of CA, Davis	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

The trace elements zinc, copper and manganese are essential for normal development in mammals. In this chapter we review the current state of knowledge on the roles of these trace elements in mammalian development, especially of the central nervous system. The effects of deficiencies of these trace elements during development in man and research animals are documented, including effects on prenatal structural morphogenesis and postnatal functional and behavioral development.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
		56
	20. SECURITY CLASS *(This page)*	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

I. INTRODUCTION

The development of the brain in mammals is a long and complex process compared to other organ systems. The brain is one of the first organs to become recognizable during embryonic development. Continued development, including increases in the number of cells, kinds of cells, and intercellular connections proceeds until long after birth in many species. This long period of development also renders the brain susceptible to developmental insults over a protracted period. Peak sensitivity corresponds to periods of rapid development or growth; thus, nutritional deficiencies are most likely to have maximum effects on those processes most active at the time of the deficiency. Deficiencies can cause neural tube defects during organogenesis, reduction in the number of neurons during the period of neuronal production, effects on glial cell numbers during glial cell proliferation, or myelination defects during the period of myelination. Even later effects on aspects of neuropsychological development are possible if the deficiency affects synaptogenesis or neuronal plasticity (see Spreen, et al., 1984 for review).

The trace elements zinc, copper and manganese have each been shown to be essential for normal development in a wide variety of mammalian species (Hurley, 1981). Defects in form or function of the central nervous system are prominent among the developmental anomalies produced by maternal deficiency of any of these elements. In this chapter we will review the overall effects of zinc, copper or manganese deficiencies during prenatal or neonatal development, demonstrating in particular the sensitivity of the developing brain to deficiencies of these trace elements.

II. OVERVIEW OF MAMMALIAN BRAIN DEVELOPMENT

Approximately one week after fertilization of a human ovum, the embryo, which consists of a hollow ball of cells called a blastula, attaches to the uterine epithelium and becomes implanted. At this stage the embryo consists of two germ layers, ectoderm and endoderm. The blastula then invaginates to form mesoderm, the third germ layer, via the process of gastrulation. The mesoderm forms between the ectoderm and the endoderm. Gastrulation is essential for neural induction, that is, the ability of the mesoderm to induce the ectoderm to form a neural plate (Saxen, 1980). In humans, neural induction takes place on day 18 of gestation.

The neural plate forms from embryonic ectoderm which overlies the mesodermal notocord. The neural plate thickens and forms a longitudinal neural groove. The plate then forms into a deep V shape and eventually the edges of the neural ectoderm come into contact and fuse. This process begins in the mid-region of the human embryo and proceeds in both rostral and caudal directions, forming the neural tube. The tube temporarily remains open at both ends, the anterior and posterior neuropores. The neuropores close at about 25 days of gestation. The formation of the neural tube from the neural plate is termed neurulation (Levi-Montalcini, 1964). Failure of the neural tube to close can lead to several malformations, depending on the location and extent of nonclosure. Lack of closure in the anterior neuropore region will result in anencephaly or exencephaly, while an open neural tube in more caudal regions constitutes spina bifida. Neurulation occurs in the third and fourth weeks of gestation in the human; closure defects therefore originate during this period (Reinis and Goldman, 1980).

Once the neural tube is formed, cells in the wall of the tube proliferate, and growth increases (Lund, 1978). Growth is accelerated at the

anterior end of the neural tube, and this region becomes divided into vesicles, the prosencephalon (forebrain), mesencephalon (midbrain) and rhombencephalon (hindbrain). During the fifth week of gestation the prosencephalon divides into the telencephalon, which will form the cerebral hemispheres, and the diencephalon, which will form the thalamus and hypothalamus and related structures. The rhombencephalon divides into the metencephalon (cerebellum and pons) and the myelencephalon (medulla oblongata) (Lund, 1978).

The fetal period begins at about eight weeks of gestation in humans, at about 13 days of gestation in the rat. Beginning at this stage, brain development is characterized by accelerated growth. Depending on the species, the peak period of brain growth may be prenatal as in the guinea pig, sheep and monkey, postnatal as in rats and rabbits, or right around birth as in pigs and man (Figure 1). Below we will provide a brief review of developmental patterns during the fetal and postnatal periods in the mammalian brain, as they have been studied in the rat and in the human.

In the developing rat brain, different regions undergo various patterns of cell division and maturation. In the cerebrum, DNA synthesis continues until about 21 days of age (Mandel, et al., 1964). Cells continue to accumulate protein and lipid after this time, as indicated by increases in cellular protein/DNA and lipid/DNA ratios. Cerebral lipid content reaches a maximum at about 65 days, and cerebral protein at 99 days of age (Fish and Winick, 1969). In the cerebellum, cell division continues until 17 days of age. After this time, differentiation of neurons occurs and actually results in a decrease in cell size as larger, more primitive cells develop into smaller, more mature cells (Fish and Winick, 1969). In the brainstem, the total number of cells increases until 14 days of age. After this there is a large increase in the protein/DNA ratio. It is likely that this change

represents not only an increase in cell size, but also the growth and myelination of neuronal processes coming from other brain regions into the brainstem (Fish and Winick, 1969). In the hippocampus, there is an increase in DNA content between 14-17 days of age. This increase is largely due to a migration of cells into the hippocampus from under the lateral ventricles. This migration occurs on day 15 in the rat (Altman and Das, 1966a; 1966b).

The pattern of development of myelination and neurotransmitters in the brain is important because these processes relate directly to the functional activity of the brain. Myelination is always preceded by multiplication of oligodendroglial cells (Tingy, 1956). Glial cells then surround nerve axons in a spiral manner. After this process is completed, deposition of lipid begins within the myelin sheath (Bunge, 1968). The bulk of total brain lipid is present as a component of myelin, and myelin is about 80% lipid. The rate of total lipid deposition can be used to measure the rate of myelin synthesis because of the very low turnover of myelin components in all species studied (LeBaron, 1970). In most species, including man, there is generally a marked increase in total lipid content and concentration during the early development of the brain (Sperry, 1962). In the rat, the rate of cholesterol deposition peaks between 15-20 days of age, about one week after the peak of DNA synthesis (Davison and Dobbing, 1968). As myelination evolves, functional components also increase rapidly. Norepinephrine and serotonin levels and monoamine oxidase activity increase rapidly until about 30 days of age, while dopamine shows little change during the same period (Hyyppä, 1971).

Much less detailed information is available on the development of the human brain (Dobbing and Sands, 1970; Winick, 1968; Howard, et al., 1969). DNA increases linearly during the prenatal period. After birth the increase in cell number slows, reaching a maximum at about 8-12 months of age (Winick, 1968). Other studies have indicated that DNA synthesis in human brains has

two peaks, the first at about 26 weeks of gestation and the second around birth (Dobbing and Sands, 1970; 1973). These authors hypothesize that the first peak represents neuronal proliferation and the second peak glial cell proliferation. In addition, DNA polymerase activity shows a similar bimodal curve in the developing human brain (Winick, 1976). Cell division stops in the cerebrum and cerebellum at approximately the same time - between 15-19 months of age (Winick, et al., 1970).

In human brain the lipid/DNA ratio rises from shortly after birth until at least 2 years of age. Thus, myelination is occurring rapidly during this period, while the rate of cell division is decreasing (Rosso, et al., 1970). Duckett and Pearse (1966; 1967; 1968; 1969), in a series of histological and histochemical studies on the human brain, found that the brain undergoes complicated biochemical changes during fetal life. Enzymes of glycolysis become evident in the second month of fetal life, while those of oxidative metabolism are demonstrable in the third month. A number of enzymes were found to have adult levels of activity by the seventh month of gestation. Acetylcholinesterase, the presence of which is thought to indicate excitability, is localized in neurons of the anterior horn of the spinal cord as early as the tenth week of embryonic life.

While the cellular and biochemical development of the human brain achieves a largely mature pattern by two years of age, critical periods for sensory functions, social relationships, and learning may persist into the second decade (see Spreen, et al., 1984 for review). Trace element deficiencies, as with other forms of malnutrition of toxic insult, may impact on the development of these functions in a direct fashion, or indirectly by affecting sensory experience, maternal behavior, or social factors (Winick, 1976).

III. DEFICIENCIES OF ZINC, COPPER AND MANGANESE IN ANIMAL DEVELOPMENT

A. ZINC

1. Prenatal effects of maternal zinc deficiency.

The embryolethality and teratogenicity of zinc deficiency was first demonstrated in the chick by Turk, et al. (1959). Other studies in the chick demonstrated that severe maternal zinc deficiency resulted in skeletal defects in offspring, including agenesis of part or all of the limbs, skull and beak deformities, dorsal curvature of the spine and shortened and fused thoracic and lumbar vertebrae (Blamberg, et al., 1960; Keinholz, et al., 1961).

The production of congenital malformations in a mammal by maternal zinc deficiency was first reported by Hurley and Swenerton (1966) in the Sprague-Dawley rat. Fetuses of zinc-deficient rats had multiple skeletal abnormalities, observed in whole fetuses and alizarin skeletal preparations. These malformations included misshapen heads, clubbed feet, fused or missing digits, micrognathia, fusions of the ribs, spinal curvature, missing caudal vertebrae and incomplete ossification of ribs, vertebrae, cranial bones and digits. There were also internal soft tissue malformations affecting the brain, heart, lungs and urogenital system (Hurley and Swenerton, 1966; Hurley, 1967). The zinc content of fetuses from zinc-deficient females was significantly lower than that of controls. Mills, et al. (1969) and Rogers, et al. (1985) have also reported congenital malformations in rats resulting from maternal zinc deficiency. Hydrocephalus, cleft palate, microphthalmia, small lungs and multiple skeletal malformations were reported in these studies. The frequency and severity of congenital malformations resulting from zinc deficiency can be correlated with the concentration of zinc in the

maternal diet. Hurley and Cosens (1974) found a decreasing frequency of fetal malformations with increasing maternal dietary zinc during gestation.

Zinc deficiency during development has also been shown to affect adversely the biochemical development of the lung and pancreas. Maturation of the lung in preparation for birth requires the synthesis of pulmonary surfactant. In a study by Vojnik and Hurley (1977), offspring of zinc-deficient females had lower lung concentrations of phosphatidylcholine and phosphatidylethanolamine than did controls, indicating a reduced production of pulmonary surfactant. Recently, lower pulmonary surfactant levels in fetuses of rats exposed to cadmium have been shown to occur due to an induced zinc deficiency (Daston, 1982). In the developing pancreas, maternal zinc deficiency resulted in decreased zymogen granulation of acinar cells near the end of gestation. In addition, procarboxypeptidase A and chymotrypsinogen, two pancreatic digestive enzymes, were each 30% lower per cell than in controls (Robinson and Hurley, 1981a). Insulin and glucagon levels in the fetus were also decreased by maternal zinc deficiency (Robinson and Hurley, 1981b).

The effect of dietary zinc deficiency on plasma zinc concentration is rapid. In rats, plasma zinc concentration is significantly decreased 6 hours after beginning a zinc deficiency regime (Hurley, et al., 1982). Thus, even shorter periods of deficiency may result in congenital malformations. When rats were fed a zinc-deficient diet for the first ten days of pregnancy, 22% of the fetuses were malformed at term; when the zinc-deficient diet was fed until day 12, 56% were malformed. When the deficient period was days 6-14, almost 50% of fetuses were malformed at term (Hurley, et al., 1971). The rapid effect of dietary zinc deficiency suggests that the pregnant rat cannot mobilize zinc from body stores in amounts sufficient to supply the developing embryo. This concept was tested by determining the effect of pregnancy on the

zinc content of maternal bone and liver in rats fed either a zinc-deficient or a control diet (Hurley and Swenerton, 1971). Even though the zinc content of fetuses from deficient females was lower than that of controls, zinc deficient females lost no more zinc from bone or liver than nonpregnant females fed the zinc-deficient diet for the same period of time. Thus, zinc-deficient pregnant rats were unable to mobilize additional zinc for their litters. The teratogenicity of dietary zinc deficiency is partially alleviated when maternal tissue catabolism is increased by simultaneously withholding dietary calcium (Hurley and Tao, 1972) or by reducing the intake of the zinc-deficient diet (Masters, et al., 1983). Alternatively, if pregnant rats are forced fed a zinc-deficient diet in amounts over that normally consumed, the number of malformations per fetus is significantly increased (Masters, et al., 1983).

2. Postnatal effects of prenatal zinc deficiency.

When pregnant rats were fed a zinc-deficient diet on days 6-14 of pregnancy, offspring had low birth weight and a high incidence of malformations. Many of the offspring were stillborn, and survival to weaning was poor (Hurley, et al., 1971). Less severe (marginal) maternal zinc deficiency during pregnancy or pregnancy and lactation also results in poor survival of offspring; studies by Hurley and Cosens (1974) and Rogers, et al. (1984) have demonstrated a positive correlation between increasing maternal dietary zinc and survival of offspring. When pregnant rats were fed a control diet throughout pregnancy and given a zinc-deficient diet after parturition, milk production was impaired and the zinc concentration of the milk was reduced. Pups suckling zinc-deficient females had low plasma zinc concentration and showed signs of zinc deficiency, including impaired growth and increased mortality (Mutch and Hurley, 1974).

Zinc deficiency during fetal or early postnatal development can also adversely affect growth and later function of lymphoid organs. In developing mice, zinc deficiency results in small thymuses (Beach, et al., 1980), depressed response to mitogens (Beach, et al., 1979), and severe reduction of serum IgM, IgG_{2A} and IgA (Beach, et al., 1980). Strikingly, deficits in circulating immunoglobulins can persist for up to three generations, even though only the first generation was exposed to zinc deficiency during prenatal development (Beach, et al., 1982).

3. Effects of zinc deficiency on brain development.

Severe zinc deprivation during embryonic and fetal development in the rat results in profound effects on virtually all derivatives of the neural tube and associated structures, including the brain, spinal cord, eyes and olfactory tract (Warkany and Petering, 1973; Adeloye and Warkany, 1976). Hurley and coworkers found major brain defects in 47% of term fetuses of dams fed a zinc deficient diet throughout pregnancy. These defects included hydrocephalus, anencephalus, hydranencephalus, and some exencephalus (Hurley, et al., 1961). Hurley and Shrader (1972) and Warkany and Petering (1972; 1973) have demonstrated that in addition to these major structural defects, microscopic lesions of the brain tissue were often present in fetuses whose heads seemed normal externally. Defects revealed by microscopic examination included tissue disorganization, missing commissures, and rosette and ductule formation.

The gross malformations of the brain seen in zinc-deficient fetuses at term (hydrocephalus, exencephalus) have been attributed to pathological closure of the aqueduct of Sylvius (Hurley and Shrader, 1972), although it has been noted that occlusion of the aqueduct could be a secondary effect of

hydrocephalus rather than the primary cause of hydrocephalus (Adeloye and Warkany, 1976).

In a study of rat embryos on day 12 of gestation, it was demonstrated that the incorporation of tritiated thymidine was lower in embryos of zinc-deficient dams than in controls (Swenerton, et al., 1969). In a subsequent study the head region was shown to be affected to a greater extent that the rest of the embryo (Eckert and Hurley, 1977). Autoradiographs demonstrated that the lower uptake of tritiated thymidine by zinc-deficient embryos occurred mainly in the developing central nervous system, and that this effect could be reversed by zinc supplementation. During this same stage of development, zinc-deficient rat embryos showed a decreased cellularity of the neural tube, which was extremely thin walled compared to controls (Swenerton, et al., 1969). The neural tube also contained more mitotic figures than were seen in controls, suggesting that cells were unable to divide normally. More recently, we have observed large numbers of pycnotic cells and cellular debris in the wall and lumen of all regions of the developing brain in zinc-deficient embryos examined on days 12-14 of gestation (Figure 2). Incorporation of tritiated thymidine into DNA is depressed in brains of zinc-deficient rat fetuses in the third trimester of gestation as well (Dreosti, et al., 1980). McKenzie and coworkers (1975) found lower brain weight and DNA and higher brain protein/DNA ratios (indicating larger cell size) in fetuses of dams fed a zinc-deficient diet during the latter third of pregnancy than controls. These studies further demonstrate that zinc deficiency can impair cell division in the developing brain. Zinc-deficient rat fetuses late in gestation also showed low brain levels of the zinc-containing enzyme alkaline phosphatase (Dreosti, et al., 1980). All neural tissues studied have been found to contain this enzyme, and it has been suggested to play a role in myelination (Cohen, 1970) and brain maturation (Cohn and Richter, 1956).

Dvergsten (1984) found retarded synaptogenesis and differentiation of cerebellar neurons in rat pups subjected to zinc deficiency during the suckling period.

Morphogenesis of the eye is sensitive to maternal zinc deficiency in the rat. Anophthalmia or microphthalmia of varying severity was noted in 38% of the fetuses of rats fed a zinc-deficient diet throughout pregnancy (Rogers, et al., 1985). Colobomata of the retina have been described in zinc-deficient fetuses by several investigators (Hurley and Shrader, 1972; Warkany and Petering, 1972; Rogers and Hurley, 1984). This defect is due to failure of the choroid fissure to close. A persistent choroid fissure prevents development of vitreous pressure within the eye; this pressure has been shown to be essential for normal expansion of the eye during later development (Coulombre, 1956). Microphthalmia can thus be the result of a persistent choroid fissure. Microphthalmic eyes from zinc-deficient fetuses often contained large retinal folds. However, histogenesis of the retina did not seem to be severely affected, as the cell layers of retinas of zinc-deficient fetuses at term were similar to controls in terms of thickness and degree of differentiation, even when retinal folding occurred (Rogers and Hurley, 1984).

Prenatal or early postnatal zinc deficiency can also result in a variety of behavioral and learning deficits. Warkany and Petering (1972) acknowledged that the microscopic lesions discovered in otherwise normal brains of zinc-deficient fetuses could probably cause functional disabilities. Halas and Sandstead (1975) and Halas, et al. (1976) fed pregnant rats a zinc-deficient diet during the third trimester of pregnancy, and tested behavior of offspring at young adulthood. Active avoidance was impaired in male offspring of these dams when they were exposed to 1 mA shock. Male offspring of dams given the zinc-deficient diet were also more sensitive to shock, which resulted in impaired learning. These effects of prenatal zinc deficiency were not seen in

female offspring. However, at 75 days of age aggression was judged to be higher in the female offspring of zinc-deficient dams than in controls. Hughes and Horsburgh (1982) cross-fostered pups from zinc-deficient dams to control dams at birth. Compared to control pups, prenatally zinc-deficient pups opened their eyes earlier and displayed greater ambulatory activity on days 28-31 of age. From day 56 on, deficient pups reared less in an exploratory box, deficient males displayed greater open-field ambulation, and deficient females had higher novelty preference. Due to higher neonatal mortality in zinc-deficient litters, it is likely that this sample was biased towards the strongest pups, which were capable of surviving until the time of testing. Oberleas, et al. (1972) also studied behavior in rat pups subjected to zinc deficiency during gestation. A control diet was fed for three weeks prior to testing. In one-way avoidance conditioning and Lashley III water maze tests it was determined that zinc-deficient pups had a slower learning rate that controls, but similar learning capacity. Postnatal zinc deficiency has been reported to impair performance on an elevated Tolman-Honzig maze (Lokken, et al., 1973) and to reduce short-term memory in adult rats (Halas and Sanstead, 1982). Halas and coworkers (1983) tested working memory, reference memory, forgetting and learning in adult rats who suffered severe zinc deficiency and/or undernutrition during the suckling period. Rats subjected to lactational zinc deficiency experienced a severe learning deficit and some working memory deficit, while pair-fed rats had only mild learning deficits compared with ad libitum fed controls. When dams were fed a diet containing 10 µg/g Zn (marginally deficient) throughout gestation and lactation, their offspring had impaired learning and/or working memory, as well as reduced areas of neuropil and neuronal layers within the hippocampus and dentate gyrus (Kawamoto and Halas, 1984).

Golub, et al. (1983; 1984) examined the impact of severe or marginal zinc deficiency during the period of rapid brain growth in the mouse. Relative brain weights of marginally zinc-deficient pups were significantly lower than controls at 10 days of age. At adulthood, behavior of developmentally zinc-deprived animals was assessed using the passive avoidance test, a test known to be a sensitive indicator of hippocampal damage. Both severely and marginally zinc-deficient pups had impaired passive avoidance performance compared to controls. In this regard, it is relevant to note that the concentration of zinc is high in the hippocampal mossy fibers, where it is contained primarily in the nerve terminals (Haug, 1967; Danscher and Zimmer, 1978). It has also been demonstrated in the rat brain that divalent zinc is preferentially taken up into mossy fiber neuropil (Howell, et al., 1984), and is released during excitation of hippocampal slices (Howell, et al., 1984; Assaf and Chung, 1984). Furthermore, in zinc-deficient adult rats, low frequency stimulation of mossy fiber axons produced synapse responses that declined abnormally in amplitude, suggesting that the heavy deposits of zinc normally found in mossy fiber boutons are important for synaptic transmission (Hesse, 1979).

In Rhesus monkeys, Sandstead, et al. (1976) fed a zinc-deficient diet from the 110th to the 150th day of pregnancy. Zinc-deficient infants were found to have increased association and prolonged contact with their mothers, increased nursing time, and decreased play activity and exploratory behavior compared to controls.

4. Mechanisms of effect of zinc deficiency.

Zinc is an essential cofactor for over 100 enzymes, both metalloenzymes and metal-enzyme complexes (Vallee, 1976; Chelebowski and Coleman, 1976),

including nucleic acid synthetic enzymes. Investigators in Vallee's laboratory, using Euglena gracilis, have provided conclusive evidence that zinc deficiency can alter DNA synthesis, chromatin structure, and cell division (Falchuk, et al., 1975; Stankiewicz et al., 1983; Mazus, et al., 1984). Studies on cellularity of the developing nervous system and tritiated thymidine incorporation into DNA, discussed in the previous section, indicate that these effects can also occur in developing mammalian tissues. Decreased activity of thymidine kinase may be involved (Dreosti and Hurley, 1975; Dreosti, et al., 1980; Duncan and Dreosti, 1975; Duncan and Hurley, 1978; Prasad and Oberleas, 1974; Record and Dreosti, 1979), although effects on RNA or protein synthesis may be more important, as a number of zinc metalloenzymes participate in the processes of transcription and translation (O'Dell, 1974).

Thus, the currently favored theory on the mechanism of action of zinc deficiency involves effects on chromosomal structure and replication, nucleic acid metabolism, and cell stability and division. However, with such a large number of enzymes depending on zinc as a cofactor, there are probably multiple biochemical lesions involved in most developmental defects caused by zinc deficiency. In addition, zinc is required for effective digestion, absorption and utilization of other nutrients, including vitamin A (Smith, et al., 1973) and protein (Hardie-Muncy and Rasmussen, 1979).

5. Zinc deficiency in humans

In the early 1960's Prasad and coworkers found symptoms of growth retardation, delayed sexual maturation and skin lesions in a group of young malnourished men in Egypt and Iran that could be attributed to a long-term nutritional zinc deficiency (Prasad, et al., 1963). Individuals with this syndrome can respond favorably to dietary zinc supplementation with signficant increases in growth rate and sexual maturation (Halsted, et al., 1972). The

occurrence of acute zinc deficiency syndromes in humans was again demonstrated in 1973 by Moynahan and Barnes, who discovered that the clinical manifestations of the potentially lethal genetic disorder acrodermatitis enteropathica were resolved with dietary zinc supplementation. Since these early observations it is now accepted that human zinc deficiency is not unusual.

While a direct link between maternal zinc deficiency and teratogenesis has not been established in humans, considerable data suggest that maternal zinc deficiency is correlated with abnormal fetal development. In several studies it has been found that mothers who give birth to anencephalic infants, or infants with spina bifida, tend to have lower plasma zinc levels at term compared with mothers who give birth to normal infants (Damyanov and Dutz, 1971; Sever and Emanuel, 1973; Bergman, et al., 1980; Stewart, et al., 1981; Cavdar, et al., 1983). In an extensive study by Jameson in 1976, low maternal zinc levels during the first and second trimester of pregnancy were correlated with an increased risk of fetal abnormalities and overall poor pregnancy outcome. Strong evidence that zinc deficiency is teratogenic in humans also can be taken from studies on the outcome of pregnancies in women with untreated acrodermatitis enteropathica. Seven pregnancies have been registered to date; two gave birth to babies with malformations, one an anencephalic fetus and the other an achrondroplastic dwarf. Two of the other pregnancies resulted in low birth weight infants (Verburg, et al., 1974; Hambidge, et al., 1975). In contrast, pregnancy outcome in women with acrodermatitis enteropathica given zinc therapy has been normal to date (Brenton, et al., 1981).

Finally, zinc deficiency secondary to chronic alcohol abuse may be one of the metabolic disorders underlying Fetal Alcohol Syndrome (Hurley, 1979). Serum zinc levels have been reported to be lower in chronic alcoholics than in non-alcoholics (Sullivan and Heaney, 1970), and low maternal plasma zinc

levels were noted in a series of alcoholic mothers who gave birth to infants with Fetal Alcohol Syndrome (Flynn, et al., 1981). It can not be said with certainty that the neurological abnormalities associated with Fetal Alcohol Syndrome are due to zinc deficiency. However, the similarity of the expression of this syndrome to that of experimental zinc deficiency in animal models strongly suggests that an alcohol-induced maternal/embryonic zinc deficiency is one of the factors underlying the abnormal development of the central nervous system in these infants.

B. COPPER

Hart and coworkers provided definitive evidence that copper is an essential component of the diet in 1928, demonstrating that both copper and iron were necessary for hemoglobin formation in rats. The necessity of copper for normal development was discovered by Bennetts, et al. (1937; 1942), who demonstrated that enzootic ataxia, a neurological disorder affecting developing lambs, could be prevented by treatment of the ewe with copper during the latter part of pregnancy.

The developing central nervous system is particularly vulnerable to copper deficiency, and neurological abnormalities are the most consistent finding with developmental copper deficiency. Therefore, we will discuss central nervous abnormalities first and then briefly consider the effects of copper deficiency on other developing tissues.

1. Effects of copper deficiency on the developing central nervous system.

In the human, only the liver has a higher concentration of copper than the brain (Smith, 1967). In man, sheep, rat and mouse there is a consistent increase in brain copper from the fetal to the neonatal to the growing young stage (Smith, 1983). Reviews of the roles of copper in the developing brain

and effects of copper deficiency have recently been published (Smith, 1983; O'Dell and Prohaska, 1983).

It has been demonstrated that enzootic ataxia in lambs is a direct result of a deficit of copper in the developing central nervous system (Barlow, et al., 1960; Schulz and Behrens, 1960; Spais, et al., 1961). Lambs with enzootic ataxia have spastic paralysis, severe incoordination, anemia and, in some cases, blindness and deafness. It is generally considered to be the same disease as "swayback" (Barlow, et al., 1960; Howell, et al., 1969; Mills and Williams, 1962). Two characteristic lesions occur in the central nervous system with this disease. The first is focal to widespread amyelination of the cerebrum (Barlow, et al., 1960; Spais, et al., 1961), and the second is progressive demyelination of the spinal cord (Barlow, et al., 1960; Barlow, 1963a, 1963b; Smith, et al., 1977). Ultrastructural defects in lambs with enzootic ataxia include a progressive loss of Nissl substance, golgi apparatus abnormalities, increased numbers of neurofibrils and abnormal neuronal and astroglial processes, including axonal degeneration (Mills and Williams, 1962; Fell, et al., 1965; Cancilla and Barlow, 1966a; 1966b; 1968; Howell, et al., 1969).

The susceptibility of the developing brain to copper deficiency has also been clearly demonstrated in the guinea pig (Everson, et al., 1967; 1968) and the rat (Carlton and Kelly, 1969; Hall and Howell, 1973; DiPaolo and Newberne, 1974; Prohaska and Wells, 1975; Zimmerman, et al., 1976; Morgan and O'Dell, 1977). Faulty myelination, via either impaired synthesis or degenerative loss of myelin, is the major lesion in these species as well. In copper-deficient guinea pigs, failure of myelination is evident throughout the brain; brains are pale and translucent with many small hemorrhagic areas (Everson, et al., 1967; 1968). In addition, cerebellar folia were often absent or abnormal. In rats that are copper deficient during gestation, lesions are found in many

areas of the brain, including the cerebral cortex, olfactory bulbs, thalamus and corpus striatum. Again, there is a general paucity of myelin throughout the brain (Carlton and Kelly, 1969; Hall and Howell, 1973; Di Paolo and Newberne, 1974; Morgan and O'Dell, 1977). Newborn rat pups from copper-deficient dams were hyperirritable, with convulsive seizures after stimulation (Di Paolo and Newberne, 1974).

The concentration of neurotransmitters in brain tissue is useful as an index of brain function. Dopamine-beta-hydroxylase (DBH) is a biochemical marker for cells producing norepinephrine (NE) or epinephrine. The adrenal form of DBH is a cuproenzyme (Friedman and Kaufman, 1965), and it is likely that the brain form is also copper-dependent. In humans the principal cell group producing NE is in the locus ceruleus; this small region also contains the highest concentration of copper in the central nervous system (Warren, et al., 1960). It has been demonstrated in vivo that the concentration of NE in the brain is lower than normal in copper-deficient rats (Morgan and O'Dell, 1977; Feller and O'Dell, 1980) and mice (Prohaska and Smith, 1982) and ataxic lambs (O'Dell, et al., 1976). Normal levels of NE in the brain can be restored by copper supplementation in the rat (Feller and O'Dell, 1980). Depressed dopamine levels have been measured in the brains of ataxic lambs (O'Dell, et al., 1976) and copper-deficient rats (Morgan and O'Dell, 1977; Feller and O'Dell, 1980).

In addition to alterations in levels of brain neurotransmitters, the density of neurotransmitter receptors is affected by copper deficiency in the rat brain. In the corpus striatum the density of high and low affinity dopamine receptors were lowered by 55% and 29%, respectively (Feller and O'Dell, 1980). Muscarinic receptor density was 50% lower than controls in the striatum; a lesser reduction occurred in the cortex and no decrease occurred

in the cerebellum. Receptor affinities were not affected by copper deficiency.

Copper deficiency can be produced by drugs as well as by diet. D-Penicillamine (DPA, dimethyl cysteine) is a copper-chelating drug which has been used in the treatment of Wilson's disease, a genetic disorder characterized by high levels of copper in tissues (Walshe, 1956). DPA produces teratogenic effects, and neural tube lesions were among the malformations seen in rats (Wiley and Joneja, 1978; Yamada, et al., 1979; Keen, et al., 1983b). These teratogenic effects have been correlated with low tissue copper levels (Keen, et al., 1983b).

There are several genetic mutants in mice which have been proposed as models of abnormal copper metabolism. The best studied are the mottled mutants; at least five mutant alleles exist at the mottled locus. One, the brindled mutant, has been studied neurologically (Hunt and Johnson, 1972; Prohaska and Smith, 1982). The neurological characteristics of this mutant are similar to those of mice with diet-induced copper deficiency, including decreases in the activity of a number of copper-dependent enzymes in the brain. Mice homozygous for the mutant gene "quaking" exhibit a severe deficit of myelin in the central nervous system and a body tremor. Depending on the strain background these mice can have low brain copper levels; in these cases supplemental copper elevates brain levels to normal and decreases the tremor frequency (Keen and Hurley, 1976). It is important to point out that in contrast to the mottled mutants, in which altered copper metabolism is a primary defect, this is not the case for the quaking mutant. Rather, the quaking mutant may be an example of how strain background can affect the expression of a mutant gene with regard to mineral metabolism (Hurley, et al., 1980).

2. Effects of copper deficiency on other developing tissues.

In addition to the central nervous system abnormalities discussed in the previous section, copper deficiency also affects adversely the development of the skeleton, the cardiovascular system and the lung.

Skeletal defects occur as a result of copper deficiency in both prenatal and postnatal life. Poorly developed bones with thin cortices were seen in lambs with enzootic ataxia, and rib fractures were common (Bennetts, 1932; 1935). As with other symptoms of this disease, bone defects could be prevented by copper supplementation of the ewe during pregnancy (Bennetts and Beck, 1942). Skeletal defects have also been described in copper-deficient calves (Cunningham, 1950; Davis, 1950) and foals (Bennetts, 1935). Copper-deficient dog pups had deformed leg bones with enlarged and hyperextended wrists and enlarged elbows. The lesions observed are consistent with impaired osteogenesis, including thinning of bone corticies, trabecular rarefaction and compressed, broadened epiphyses and metaphyses (Baxter, 1951; Baxter and Van Wyk, 1953; Baxter, et al., 1953). Similar defects were found in offspring of copper-deficient swine (Teague and Carpenter, 1951; Lahey, et al., 1952; Follis, et al., 1955; Follis, 1958). Copper-deficient chicks, in contrast, tend to have severe hypoplasia of the long bones (Baxter and Van Wyk, 1953; Baxter, et al., 1953; Follis, 1958; Carlton and Henderson, 1964). Recently, a mutant line of chickens highly susceptible to scoliosis has been shown to respond to supplemental dietary copper with a decreased expression of the lesion (Opsahl, et al., 1984).

Cardiac hemorrhages were noted in lambs with enzootic ataxia (Bennetts, 1932), and fibrotic degeneration of the myocardium was noted in cattle with "falling disease", a copper deficiency disease (Bennetts, et al., 1948). Newborn offspring of copper-deficient rats suffer hemorrhages of the heart,

aorta and other large vessels, as well as small lesions throughout the cardiovascular system (O'Dell, et al., 1961). Copper-deficient guinea pigs (Everson, et al., 1967) and rabbits (Nystrom, 1965; 1968) have similar lesions.

When offspring of rats fed a copper-deficient diet throughout pregnancy and lactation were subsequently fed a copper-deficient diet for an additional 6-10 weeks after weaning, their lungs had 35% larger alveolar spaces than did controls, indicating an emphysemic condition (O'Dell, et al., 1978).

3. Mechanisms of effect of copper deficiency.

There are a number of copper-dependent enzymes in the brain that are affected by copper deficiency (O'Dell and Prohaska, 1983). Reduced activity of cytochrome oxidase, the terminal enzyme in the mitochondrial electron transport chain, has often been cited as the basis for impaired myelination. There is good evidence that cytochrome oxidase activity is decreased in the brains of copper-deficient animals (Howell, et al., 1959; Mills and Williams, 1962; Barlow, 1963b; Gallagher, et al., 1973). If cytochrome oxidase activity is sufficiently reduced, the resultant respiratory constraint could lead to demyelination (Lumsden, 1950). It is unclear, however, that the extent of depletion of activity in brains of copper-deficient animals is enough to lead to a significant respiratory constraint.

Lysyl oxidase is a copper-dependent enzyme involved in the crosslinking of collagen and elastin (O'Dell, 1981). This process is essential for the maintenance of vascular integrity. Thus, reduced lysyl oxidase activity may play a role in the vascular abnormalities of the brain and other organs seen in dietary or genetic copper deficiency. Low activity of this enzyme is also likely to be involved in impaired osteogenesis occurring with copper deficiency.

Copper-zinc superoxide dismutase is a cupro-zinc enzyme found in the cytosol. It catalyzes the dismutation of superoxide anions, producing hydrogen peroxide. This reaction protects lipids in membranes and elsewhere from free radical-induced peroxidative damage. It is estimated that as much as 25% of the total copper pool in the rat brain is associated with copper-zinc superoxide dismutase (O'Dell and Prohaska, 1983). Activity of this enzyme is reduced by 30-35% in brains of second-generation copper-deficient rats (Prohaska and Wells, 1975; Morgan and O'Dell, 1977). However, there is currently no evidence of increased lipid peroxidation in the brains of copper deficient animals (Prohaska and Wells, 1975).

4. Copper deficiency in humans

The extent to which copper deficiency contributes to teratogenesis in human beings is not clear. Morton, et al. (1976) have reported a significant correlation between low copper in drinking water and neural tube defects in south Wales. Wald and Hambidge (1977), however, were unable to demonstrate a relationship between maternal serum copper concentration and the incidence of anencephaly. While maternal copper deficiency has not been clearly linked to an increased risk of birth defects, the fact that many individuals are thought to be marginal with regard to dietary copper intake (Klevay, 1982) shows the importance of assessing the possible effects of maternal hypocupremia on fetal outcome.

In contrast to the lack of data on the prevalence of maternal copper deficiency, there have been numerous reports of copper deficiency in infants and children. In the majority of cases it has been assumed that the copper deficiency arises postnatally, usually the result of prolonged consumption of cow's milk, a food low in copper, or as a consequence of severe undernutrition coupled with diarrhea. Typically, these individuals are characterized by

hematological and skeletal disorders which are correctable by copper therapy (Al-Rashid and Spangler, 1971; Graham and Cordano, 1976). To the authors' knowledge there has been no documentation of permanent neurological damage associated with neonatal copper deficiency. One complication of such studies will be that the child will often be deficient in several nutrients in addition to copper. However, despite this problem, long term follow-up studies on possible behavioral abnormalities in individuals diagnosed as being copper deficient need to be done.

While abnormal brain development due to perinatal dietary copper deficiency has not been clearly shown for humans, it is well recognized that one of the phenotypic expressions of Menkes' disease in man is abnormal central nervous system development. Menkes' disease, an x-linked genetic disorder first described in 1962 by Menkes and coworkers, is characterized by myoclonic seizures, psychomotor deficiencies, abnormal head growth, severe mental retardation, hypothermia, connective tissue defects and failure to thrive. Death often occurs before 3 years of age. Danks and coworkers (1972) first demonstrated an abnormality of copper metabolism in infants with this syndrome. Findings in these individuals include low serum copper and ceruloplasmin levels, and abnormal tissue binding of copper which results in a generalized defect of copper transport. Unfortunately, despite increased serum copper and ceruloplasmin levels with copper therapy, the long-term prognosis for these patients has not been greatly improved. One possible explanation for this failure of copper therapy is that it is started postnatally, when the disease is already in an advanced state, and that reversal does not occur. The demonstration of abnormal copper metabolism in utero in the Menkes fetus (Horn, 1983) supports this idea. Thus, it is possible that prenatal supplementation may be required for efficacious treatment.

Specific brain pathology associated with Menkes' disease includes a paucity of myelin and extensive neuronal necrosis. It is not clear at present to what extent the severe hypomyelination associated with the disease results from demyelination as opposed to amyelination. Current data would suggest that the principal problem is one of amyelination, however, the biochemical lesion(s) responsible for either the amyelination or demyelination have not been identified. Localized ischemic damage which occurs as a result of the vascular defects associated with copper deficiency may explain some of the problem. For an extensive review on the pathology of Menkes' disease the reader is directed to the review by Smith (1981).

It should be pointed out here that an excess of copper can also affect brain development and function. Severe neuropathology resulting from prolonged copper toxicosis is evident from studies on patients with Wilson's disease, an autosomal recessive disorder resulting in copper loading. The genetic defect in these individuals involves a block in the normal hepatic excretion of copper into the bile, and subsequently the gastrointestinal tract. Over a prolonged period of time liver copper levels increase, resulting in hepatic damage. If copper levels reach a critical point there can be an acute necrosis of a large number of copper-rich hepatocytes, the result being a sudden release of copper into plasma. This high level of copper in the plasma can in turn result in an acute hemolytic anemia, often the first clinical manifestation of the disease. However, prior to a hemolytic crisis, there can be a slow leakage of copper from the liver into the plasma and some of this copper can be taken up by extrahepatic tissue such as the brain. Excess copper accumulation in the brain can result in neurological disorders which particularly affect the motor system. For a detailed description of the neural pathology, the reader is directed to the chapter by Ordogh and coworkers (this volume).

C. MANGANESE

1. Teratogenic effects of prenatal manganese deficiency.

The most prominent effect of manganese deficiency during development is irreversible congenital ataxia, characterized by lack of coordination, loss of equilibrium, head retraction and tremor (Hurley, et al., 1958). This effect has been demonstrated in the chick (Caskey, et al., 1944), rat (Shils and McCollum, 1943), mouse (Erway, et al., 1966) and guinea pig (Everson, et al., 1959). In addition, congenital ataxia occurs in a number of genetic mutants of manganese metabolism, including the pallid mouse (Lyon, 1951; 1953) and screwneck mink (Erway and Mitchell, 1973). Erway and coworkers (Erway, et al., 1966; 1970) have demonstrated that ataxia results from abnormal development of the otoliths, calcified structures in the vestibular portion of the inner ear. Thus, the congenital ataxia of manganese deficiency does not involve a neurological defect. Ataxia in offspring of manganese-deficient animals or manganese mutants can be prevented by manganese supplementation during pregnancy (Hurley and Everson, 1963; Erway, et al., 1966; 1971; 1973).

The importance of manganese for normal development of the skeleton was first shown by Lyons and Insko (1937), who observed chondrodystrophy in chicks from manganese-deficient hens. A similar effect occurs in rats, with offspring showing a disproportionate growth of the skeleton, including shortening of the long bones and ulnar deviation (Hurley, et al., 1961a). Manganese deficiency in rats also produces a doming of the skull, largely due to a shortening of the basal portion of the skull (Hurley, et al., 1961c).

2. Effects of manganese deficiency on development of the brain.

Data on adverse effects of manganese deficiency on the development of the brain are few at present. When offspring of manganese-deficient rats were fed a manganese-deficient diet, their threshold for electroshock seizures was significantly lower than controls (Hurley, et al., 1961b). There were also changes in the maximal electroshock seizure pattern, indicating that brain excitability and convulsability were greater than normal in ataxic manganese-deficient rats. Later experiments showed that this increase in convulsability was due to manganese deficiency and not the presence of ataxia (Hurley, et al., 1963).

Recently, Baly, et al. (1984) have demonstrated that prenatal manganese deficiency can adversely affect postnatal carbohydrate metabolism in rats. On days 1 and 2 postpartum, plasma glucose concentrations were lower in deficient pups than in controls. Glucose is the energy source for the brain, and this period coincides with the period of rapid brain growth in the rat. However, effects on brain growth during this period have not been examined.

3. Mechanism of effect of manganese deficiency.

The biochemical lesion underlying the increase in convulsability in manganese-deficient rats is not known at present; indeed, the biological functions of manganese in the brain are not well understood. Manganese is thought to be involved in the activation of brain glutamine synthase (Wedler, et al., 1982), UDP-galactose : GN_2 ganglioside galactosyltransferase (Yip and Dain, 1970), catechol o-methyl transferase (Tagliamonte, et al., 1970) and adenylate cyclase (Katz and Tenehouse, 1973). None of the above enzyme systems have been studied under conditions of dietary manganese deficiency,

thus, it is not known if a disturbance in any of the above pathways may explain some of the neurological lesions seen with manganese deficiency.

Manganese may also be directly involved in biogenic amine metabolism via complex formation. If this is true, then normal cellular manganese concentrations may be essential for normal binding, transport, and storage of catechols and ethanolamines (Papavasiliou, 1981).

Skeletal and otolith abnormalities produced by manganese deficiency are thought to be due to a defect in the synthesis of mucopolysaccharides (Leach and Muenster, 1962; Hurley, et al., 1968; Shrader, et al., 1973). Leach and colleagues (Leach and Lilburn, 1978) have demonstrated that manganese is a cofactor for glycosyl transferase, which functions in the synthesis of mucopolysaccharides. It is likely that reduced activity of this enzyme in manganese-deficient animals plays a role in both otolith and skeleton defects.

The activity of manganese superoxide dismutase is significantly lower in tissues of manganese deficient rats than in controls. Low activity of the enzyme has been correlated to an increased rate of tissue lipid peroxidation and cell membrane damage (Zidenberg-Cherr, et al., 1983; 1985). These changes have not been reported in very young animals, thus, a reduction in the activity of manganese superoxide dismutase may not contribute significantly to to prenatal and early postnatal brain structural defects. Altered lipid and protein metabolism may also occur as a result of manganese deficiency (Keen, et al., 1985) but again this has not been demonstrated to be a factor in the developing brain.

4. Manganese deficiency in humans.

The importance of the observations on the effects of manganese deficiency on brain function in experimental animals is underscored by the report by Papavasiliou and coworkers (1979), who found that whole blood manganese was

significantly lower in epileptics than in controls. Furthermore, blood manganese concentration correlated with the frequency of seizures. Patients with only a few seizures a year had blood manganese levels similar to those of controls, while those with frequent seizures (1-3/month) had low concentrations. Blood manganese levels were not correlated with blood levels of the anticonvulsive drugs taken by these patients, indicating that the low blood manganese was not attributable to drug therapy. Tanaka (1977) has also reported that one-third of children with convulsive disorders of unknown origin had whole-blood manganese concentrations significantly lower than normal. However, these results have only been presented as abstracts. Currently it is not known if the low blood manganese levels found in some epileptics is due to a primary manganese deficiency, which in turn results in the neurological abnormalities, or rather if the low levels are a consequence of the disease.

There has been one case reported of dietary manganese deficiency in a human subject (Doisey, 1972). This volunteer developed manganese deficiency following its accidental omission from a purified diet which was being used to study the effects of vitamin K deficiency. Signs which were thought to be associated with manganese deficiency included weight loss, reddening of his black hair, reduced growth of hair and nails, dermatitis, hypercholesterolemia, and inability to elevate depressed clotting proteins in response to vitamin K administration. Neurological abnormalities were not reported in this individual. While this is the only report of frank manganese deficiency in man, several other investigations suggest that suboptimal manganese status may not be unusual. Poor manganese status, as defined by low hair and plasma manganese levels, has been reported in children suffering from the inborn errors of metabolism maple syrup urine disease and phenylketonuria, even though dietary intake of manganese was similar to that of control

children (Hurry and Gibson, 1982). Liver and heart manganese levels have been reported to be lower than normal in children suffering from protein-calorie malnutrition (Lehmann, et al., 1971).

Finally, it has been suggested that manganese deficiency may be related to hydralazine disease, a syndrome that simulates systemic lupus erythematosis or rheumatoid arthritis. Hydralazine disease occurs in some people taking the antihypertensive drug, hydralazine, and it has been reported to be responsive to manganese therapy (Comens, 1956). Manganese supplementation also reduces some aspects of the disease in dogs and rats given hydralazine (Comens, 1956; Hurley, et al., 1963). Thus, manganese deficiency may arise as the result of some drug therapies.

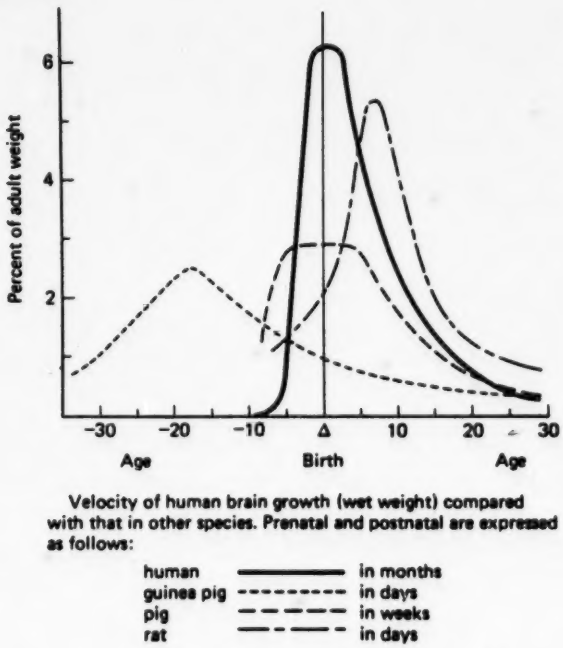
While, at the present time, manganese deficiency has only been implicated in a few human disorders, this may be due in part to lack of a practical method for assessing an individual's manganese status. We have recently found that whole blood manganese can be a useful indicator of whole body manganese status, with low blood levels of manganese in manganese deficient rats reflecting low levels of the element in soft tissue (Keen, et al., 1983a). These observations support the idea that blood manganese levels are a useful indicator of soft tissue manganese levels, and not just a reflection of recent diet history.

It should be pointed out that in addition to manganese deficiency, manganese toxicity can also affect brain function, and there are numerous reports on the effects of manganese toxicity on brain function in humans who chronically inhale large amounts of airborne manganese, a situation which occurs in manganese mines, steel mills and some chemical industries. Excess manganese enters the body mainly as oxide dust via the lungs and also via the gastrointestinal tract from the contaminated environment (Cotzias, 1958). The lungs apparently act as a depot from which the manganese is continuously

absorbed. Manganese poisoning is characterized by a severe psychiatric disorder (locura manganica) resembling schizophrenia, followed by a permanently crippling neurological disorder clinically similar to Parkinson's disease. The presence of elevated tissue manganese levels is thus not necessary for the continuance of the neurological manifestations of the disease, and metal chelation therapy is unlikely to secure their remission (Cotzias, et al., 1968).

Recently the neurotoxicity resulting from manganese has received increasing attention because of the use of manganese-containing compounds in gasoline as a replacement for lead (Ter Harr, et al., 1975). In addition it has been reported that children living in urban areas can have elevated blood manganese levels (Joselow, et al., 1978). It is not clear at the present time if manganese toxicity during early development is a problem in man; however, the observation that the neurological lesions resulting from manganese toxicity are permanent shows the importance of not oversupplying this element to the infant.

From Dobbing, 1972.



Velocity of human brain growth (wet weight) compared with that in other species. Prenatal and postnatal are expressed as follows:

Figure 1. Rate curves of brain growth in relation to birth in different species. Values are calculated at different time intervals for each species.

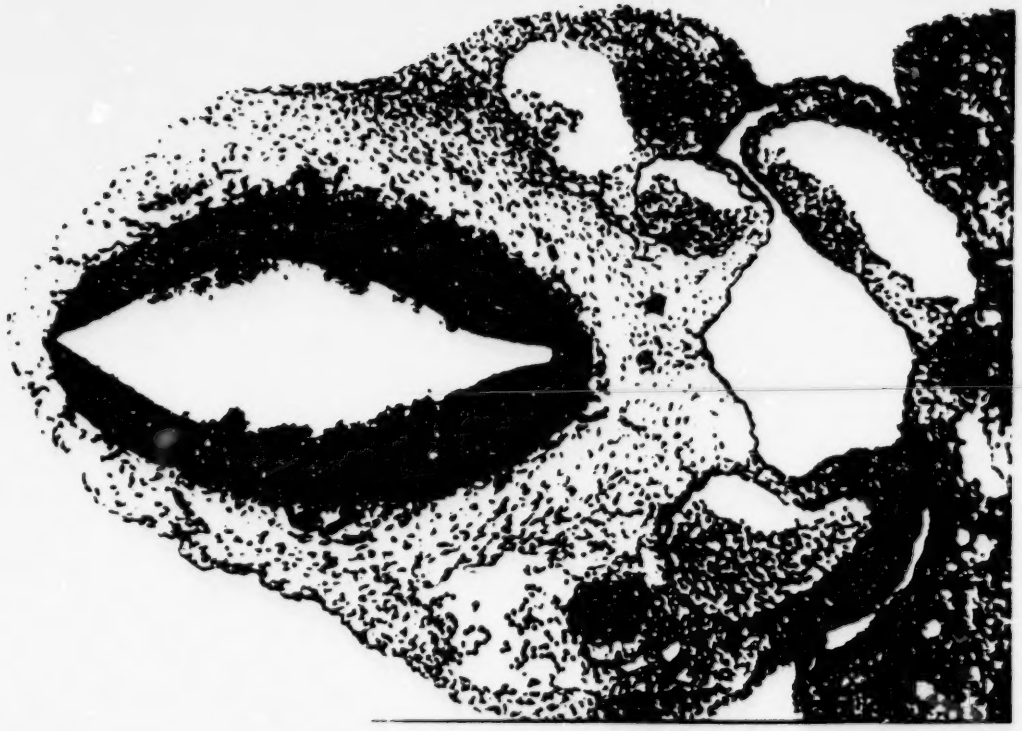


Figure 2. Cross section through the brain of a zinc-deficient fetus at gestation day 14. Note large number of pycnotic cells in the neuroepithelium.

REFERENCES

Adeloye A, Warkany J (1976): Experimental congenital hydrocephalus. Child's Brain 2:325-360.

Al-Rashid RA, Spangler J (1971): Neonatal copper deficiency. N Engl J Med 285:841-843.

Altman J, Das GP (1966a): Autoradiographic and histological studies of postnatal neurogenesis. I. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in neonate rats, with special reference to postnatal neurogenesis in some brain regions. J Comp Neurol 126:337-390.

Altman J, Das GP (1966b): Autoradiographic and histological studies of postnatal neurogenesis. II. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in infant rats, with special reference to postnatal neurogenesis in some brain regions. J Comp Neurol 128:431-474.

Assaf SY, Chung SH (1984): Release of endogenous Zn^{2+} from brain tissue during activity. Nature 308:734-736.

Baly DL, Keen CL, Curry DL, Hurley LS (1984): Effects of manganese deficiency on carbohydrate metabolism. In: "Trace Element Metabolism in Man and Animals (TEMA-5)", In Press.

Barlow RM (1963a): Further observations on swayback. I. Transitional pathology. J Comp Pathol 73:51-60.

Barlow RM (1963b): Further observations on swayback. II. Histochemical localization in the central nervous system. J Comp Pathol 73:61-67.

Barlow RM, Purves D, Butler EJ, MacIntyre IJ (1960): Swayback in South-East Scotland. II. Clinical, pathological and biochemical aspects. J Comp Pathol 70:411-427.

Baxter JH (1951): Bone disorder in copper-deficient puppies. Am J Physiol 167:766.

Baxter JH, Van Wyk JJ (1953): A bone disorder associated with copper deficiency. I. Gross morphological, roentgenological, and chemical observation. Bull Johns Hopkins Hosp 93:1-23.

Baxter JH, Van Wyk JJ, Follis RH Jr (1953): A bone disorder associated with copper deficiency. II. Histological and chemical studies on the bones. Bull Johns Hopkins Hosp 93:25-39.

Beach RS, Gershwin ME, Hurley LS (1979): Altered thymic structures and mitogen responsiveness in postnatally zinc-deprived mice. Dev Comp Immunol 3:725-738.

Beach RS, Gershwin ME, Hurley LS (1982): Gestational zinc deprivation in mice: persistence of immunodeficiency for three generations. Science 218:469-471.

Beach RS, Gershwin ME, Makishima RK, Hurley LS (1980): Impaired immunologic ontogeny in postnatal zinc deprivation. J Nutr 110:805-815.

Bennetts HW (1932): Enzootic ataxia of lambs in Western Australia. Aust Vet J 8:137-142, 183-184.

Bennetts HW (1935): The prevention of so-called 'rickets' in lambs and foals. J Dept Agric W Aust 12:305-310.

Bennetts HW, Beck AB (1942): Enzootic ataxia and copper deficiency of sheep in Western Australia. Austr Counc Sci Ind Res Bull 147:1-52.

Bennetts HW, Beck AB, Harley R (1948): The pathogenesis of "falling disease": studies of copper deficiency in cattle. Austr Vet J 24:237-244.

Bennetts HW, Chapman FE (1937): Copper deficiency in sheep in Western Australia: a preliminary account of the aetiology of enzootic ataxia of ewes. Aust Vet J 13:138-149.

Bergmann KE, Makosch E, Tews KH (1980): Abnormalities of hair zinc concentration in mothers of newborn infants with spina bifida. Am J Clin Nutr 33:2145-2150.

Blamberg DL, Blackwood WB, Supplee WC, Combs CF (1960): Effect of zinc deficiency in hens on hatchability and embryonic development. Prc Soc Exp Biol Med 104:217-220.

Brante G (1949): Studies on lipids in nervous system with special reference to quantitative chemical determination and topical distribution. Acta Physiol Scand (Suppl 63) 18:1-189.

Brenton DP, Jackson MJ, Young A (1981): Two pregnancies in a patient with acrodermatitis enteropathica treated with zinc sulphate. Lancet ii:500.

Bunge RP (1968): Glial cells and the central myelin sheath. Physiol Rev 48:197-251.

Cancilla PA, Barlow RM (1966a): Structural changes of the central nervous system in swayback (enzootic ataxia) of lambs. I. Electron microscopy of the cerebral lesion. Acta Neurophathol 6:251-259.

Cancilla PA, Barlow RM (1966b): Structural changes of the central nervous system in swayback (enzootic ataxia) of lambs. II. Electron microscopy of the lower motor neuron. Acta Neuropathol 6:260-265.

Cancilla PA, Barlow RM (1968): Structural changes of the central nervous system in swayback (enzootic ataxia) of lambs. IV. Electron microscopy of the white matter of the spinal cord. Acta Neuropathol 11:294-300.

Carlton WW, Henderson W (1964): Skeletal lesions in experimental copper deficiency in chickens. Avian Dis 8:48-55.

Carlton WW, Kelly WA (1969): Neural lesions in the offspring of female rats fed a copper-deficient diet. J Nutr 97:42-52.

Caskey CD, Norris LC, Heuser GF (1944): A chronic congenital ataxia in chicks due to manganese deficiency in the maternal diet. Poult Sci 23:516-520.

Cavdar AO, Babacan E, Asik S, Arcasoy A, Ertem V, Himmetoglu O, Baycu T, Akar N (1983): Zinc levels of serum, plasma, erythrocytes and hair in Turkish women

with anencephalic babies. In Prasad A (ed): "Zinc Deficiency in Human Subjects." New York: Alan R. Liss, Inc., pp 99-106.

Chelebowski JF, Coleman JE (1976): Zinc and its role in enzymes. In Sigel H (ed): "Metal Ions in Biological Systems, Vol 6." New York: Marcel Dekker, pp 1-140.

Cohen SR (1970): Phosphatases. In Lajtha A (ed): "Handbook of Neurochemistry, Vol 3. Metabolic Reactions in the Nervous System." New York: Plenum Press, pp 87-137.

Cohn P, Richter D (1956): Enzymic development and maturation of the hypothalamus. J Neurochem 1:166-172.

Comens P (1956): Manganese depletion as an etiologic factor in hydralazine disease. Am J Med 20:944-945.

Cotzias GC, Greenough JJ (1958): The high specificity of the manganese pathway through the body. J Clin Invest 37:1298-1305.

Cotzias GC, Horiuchi K, Fuenzalida S, Mean I (1968): Chronic manganese poisoning: clearance of tissue manganese concentrations with persistence of the neurological picture. Neurology 18:376-382.

Coulombre A (1956): The role of intraocular pressure in the development of the chick eye. I. Control of eye size. J Exp Zool 133:211-225.

Cummings JN, Goodwin H, Woodward EM, Curzon G (1958): Lipids in the brain of infants and children. J Neurochem 2:289-294.

Cunningham IJ (1950): Copper and molybdenum in relation to diseases of cattle and sheep in New Zealand. In McElroy WD, Glass B (eds): "A Symposium on Copper Metabolism." Baltimore: Johns Hopkins Press, pp 246-273.

Damyanov I, Dutz W (1971): Anencephaly in Shiraz, Iran. Lancet i:82.

Danks DM, Campbell PE, Stevens BJ, Mayne V, Cartwright E (1972): Menkes kinky hair syndrome: an inherited defect in copper absorption with widespread effects. Pediatrics 50:188-201.

Danscher G, Zimmer J (1978): An improved Tim sulphide silver method for light and electron microscopic localization of heavy metals in biological tissues. Histochemistry 55:27-40.

Daston GP (1982): Fetal zinc deficiency as a mechanism for cadmium induced toxicity to the developing rat lung and pulmonary surfactant. Toxicology 24:55-63.

Davis GK (1950): The influence of copper on the metabolism of phosphorus and molybdenum. In McElroy WD, Glass B (eds): "A Symposium on Copper Metabolism." Baltimore: Johns Hopkins Press, pp 216-229.

Davison AN, Dobbing J (1968): The developing brain. In Davison AN, Dobbing J (eds): "Applied Neurochemistry." Oxford: Scientific Publications, pp 253-286.

Di Paolo RV, Newberne PM (1974): Copper deficiency and the central nervous system myelination in the rat: Morphological and biochemical studies. J Neuropathol Exp Neurol 33:226-236.

Dobbing J (1972): Vulnerable periods of brain development. In Elliott K, Knight J (eds): "Lipids, Malnutrition and Developing Brain, Ciba Foundation Symposium." Amsterdam: Elsevier, pp 9-29.

Dobbing J (1976): Vulnerable periods in brain growth and somatic growth. In Roberts DF, Thomson AM (eds): "The Biology of Human Fetal Growth." London: Taylor and Francis, pp 137-147.

Dobbing J, Sands J (1970): Timing of neuroblast multiplication in developing human brain. Nature 226:639-640.

Dobbing J, Sands J (1973): Quantitative growth and development of human brain. Arch Dis Child 48:757-767.

Dodge PR, Prensky AL, Feigin RD (1975): "Nutrition and the Developing Nervous System." St. Louis: Mosby.

Doisy E Jr (1972): Micronutrient controls of biosynthesis of clotting proteins and cholesterol. In Hemphill D (ed): "Trace Substances in Environmental Health, Vol VI." Columbia MO: Univ of Missouri, pp 193-199.

Dreosti IE, Hurley LS (1975): Depressed thymidine kinase activity in zinc deficient rat embryos. Proc Soc Exp Biol Med 150:161-165.

Dreosti IE, Record IR, Manuel JJ (1980): Incorporation of ^{3}H-thymidine into DNA and the activity of alkaline phosphatase in zinc-deficient fetal rat brains. Biol Trace Element Res 2:21-29.

Duckett S, Pearse AGE (1966): The chemo-architectronic patterns of the cerebral cortex of the embryonic and fetal human brain. Proc 5th Intl Congr of Neuropathology, Excerpta Medica Foundation, Intl Congr Series 100:738-739.

Duckett S, Pearse AGE (1967): Monoamine oxidase cells in the developing human cortex. Rev Can Biol 26:173-174.

Duckett S, Pearse AGE (1968): The cells of Cajal-Retzius in the developing human brain. J Anat 102:183-187.

Duckett S, Pearse AGE (1969): Histoenzymology of the developing human spinal cord. Anat Rec 163:59-66.

Duncan JR, Dreosti IE (1975): A proposed site of action for zinc in DNA synthesis. J Comp Pathol 86:81-85.

Duncan JR, Hurley LS (1978): Thymidine kinase and DNA polymerase activity in normal and zinc deficient developing rat embryos. Proc Soc Exp Biol Med 159:39-43.

Dvergsten CL (1984): Retarded synaptogenesis and differentiation of cerebellar neurons in zinc-deficient rats. In Frederickson CJ, Howell GA, Kasarskis EJ

(eds): "The Neurobiology of Zinc. Part B: Deficiency, Toxicity, and Pathology." New York: Alan R. Liss, Inc., pp 17-31.

Erway L, Fraser A, Hurley LS (1971): Prevention of congenital otolith defect in pallid mutant mice by manganese supplementation. Genetics 67:97-108.

Erway L, Hurley LS, Fraser A (1966): Neurological defect: manganese in phenocopy and prevention of a genetic abnormality of inner ear. Science 152:1766-1768.

Erway L, Hurley LS, Fraser A (1970): Congenital ataxia and otolith defects due to manganese deficiency in mice. J Nutr 100:643-654.

Erway LC, Mitchell SE (1973): Prevention of otolith defect in pastel mink by manganese supplementation. J Hered 64:111-119.

Everson GJ, Hurley LS, Geiger JF (1959): Manganese deficiency in the guinea pig. J Nutr 68:49-57.

Everson GJ, Shrader RE, Wang TI (1968): Chemical and morphological changes in the brains of copper-deficient guinea pigs. J Nutr 96:115-125.

Everson GJ, Tsai H, Wang T (1967): Copper deficiency in the guinea pig. J Nutr 93:533-540.

Falchuk KH, Fawcett DW, Vallee BL (1975): Role of zinc in cell division of Euglena gracilis. Biochemistry 23:42-47.

Fell PF, Mills CF, Boyne R (1965): Cytochrome oxidase deficiency in the motor neurons of copper-deficient lambs: a histochemical study. Res Vet Sci 6:170-177.

Feller DJ, O'Dell BL (1980): Dopamine and norepinephrine in discrete areas of the copper-deficient rat brain. J Neurochem 34:1259-1263.

Fish I, Winick M (1969): Cellular growth in various regions of the developing rat brain. Pediat Res 3:407-412.

Flynn A, Martier SJ, Sokol RJ (1981): Zinc status of pregnant alcoholic women: a determinant of fetal outcome. Lancet i:572.

Follis R (1958): "Deficiency Disease." Springfield, IL: Thomas, pp 56-64.

Follis RH Jr, Bush JA, Cartwright GE, Wintrobe MM (1955): Studies on copper metabolism. XVIII. Skeletal changes associated with copper deficiency in swine. Bull Johns Hopkins Hosp 97:405-414.

Friedman S, Kaufman S (1965): 3,4-Dihydroxyphenylethylamine β-hydroxylase. Physical properties, copper content and role of copper in the catalytic activity. J Biol Chem 240:4763-4773.

Gallagher CH, Reeve VE, Wright R (1973): Copper deficiency in the rat. Effect on the ultrastructure of hepatocytes. Austr J Exp Biol Med Sci 51:181-189.

Golub AM, Gershwin ME, Vijayan VK (1983): Passive avoidance performance of mice fed marginally or severely zinc deficient diets during post-embryonic brain development. Physiol Behav 30:409-413.

Golub MS, Keen CL, Vijayan VK, Gershwin ME, Hurley LS (1984): Early development of brain and behavior in mice fed a marginally zinc deficient diet. In "The Neurobiology of Zinc. Part B: Deficiency, Toxicity, and Pathology." New York: Alan R. Liss, Inc., pp 65-75.

Graham GG, Cordano A (1976): Copper deficiency in human subjects. In Prasad, AS (ed): "Trace Elements in Human Health and Disease. Vol. 1. Zinc and Copper." pp 263-272.

Halas ES, Sandstead HH (1975): Some effects of prenatal zinc deficiency on behavior of the adult rat. Ped Res 9:94-97.

Halas ES, Sandstead HH (1981): Short-term memory deficits in adult rats due to marginal zinc deficiency during gestation and lactation. Fed Proc 40:839.

Halas ES, Rowe MC, Johnson OR, McKenzie JM, Sandstead HH (1976): Effects of intrauterine zinc deficiency on subsequent behavior. In Prasad AS (ed): "Proceedings of the International Symposium on Trace Elements in Human Health and Disease." Nutrition Foundation Monograph, Academic Press, pp 327-343.

Halas ES, Eberhardt MJ, Diers MA, Sandstead HH (1983): Learning and memory impairment in adult rats due to severe zinc deficiency during lactation. Physiol Behav 30:371-381.

Hall GA, Howell J McC (1973): Lesions produced by copper deficiency in neonate and older rats. Brit J Nutr 29:95-104.

Halsted JA, Ronaghy HA, Abadi P, Haghshenass M, Amirhakima GH, Banakat RM, Reinhold JG (1972): Zinc deficiency in man: The Shiraz experiment. Am J Med 53:277-284.

Hambidge KM, Neldner KH, Walravens PA (1975): Zinc, acrodermatitis enteropathica, and congenital malformations. Lancet i:577-578.

Hardie-Muncy DA, Rasmussen AI (1979): Interrelationships between zinc and protein level and source in weanling rats. J Nutr 109:321-329.

Hart EB, Steenbock H, Waddell J, Elvehjem CA (1928): Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. J Biol Chem 77:797-812.

Haug FM (1967): Electronmicroscopial localization of the zinc in hippocampal mossy fiber synapses by a modified silver procedure. Histochemie 8:355-368.

Hesse GW (1979): Chronic zinc deficiency alters neuronal function of hippocampal mossy fibers. Science 205:1005-1007.

Horn N (1983): Menkes' x-linked disease: prenatal diagnosis and carrier detection. J Inher Metab 6 Suppl 1: 59-62.

Howard E, Granoff DM, Bujnovsky (1969): DNA, RNA, and cholesterol increases in cerebrum and cerebellum during development of the human fetus. Brain Res 14:697-706.

Howell GA, Welch MG, Frederickson CJ (1984): Stimulation-induced uptake and release of zinc in hippocampal slices. Nature 308:736-738.

Howell J McC, Davison AN (1959): The copper content and cytochrome oxidase activity of tissues from normal and swayback lambs. Biochem J 72:365-368.

Howell J McC, Davison AN, Oxberry J (1969): Observations on lesions in the white matter of the spinal cord of swayback sheep. Acta Neuropathol 12:33-41.

Hughes RN, Horsburgh RJ (1982): Some behavioral effects of prenatal marginal zinc deficiency in young rats. Nutr Res 2:513-520.

Hunt DM, Johnson DR (1972): An inherited deficiency in noradrenaline biosynthesis in the brindled mouse. J Neurochem 19:2811-2819.

Hurley LS (1967): Studies on nutritional factors in mammalian development. J Nutr 91:27-39.

Hurley LS (1979): The Fetal Alcohol Syndrome: possible implications of nutrient deficiencies. In "Alcohol and Nutrition." Research Monograph 2. National Institute on Alcohol Abuse and Alcoholism. U.S. Government Printing Office. Stock number 017-024-00954-7, pp 367-382.

Hurley LS (1981): Teratogenic aspects of manganese, zinc, and copper nutrition. Physiol Revs 61:249-295.

Hurley LS, Cosens G (1974): Reproduction and prenatal development in relation to dietary zinc level. In Hoekstra WG, Suttie JW, Ganther HE, Mertz W (eds): "Trace Element Metabolism in Animals - 2." Baltimore: University Park Press, pp 516-518.

Hurley LS, Everson GJ (1963): Influence of timing of short-term supplementation during gestation on congenital abnormalities of manganese-deficient rats. J Nutr 79:23-27.

Hurley LS, Everson GJ, Geiger JF (1958): Manganese deficiency in rats: congenital nature of ataxia. J Nutr 66:309-320.

Hurley LS, Everson GJ, Wooten E, Asling CW (1961a): Disproportionate growth in offspring of manganese-deficient rats. I. The long bones. J Nutr 74:274-281.

Hurley LS, Gordon P, Keen CL, Merkhofer L (1982): Circadian variation in rat plasma zinc and rapid effect of dietary zinc deficiency. Proc Soc Exp Biol Med 170:48-52.

Hurley LS, Gowan J, Swenerton H (1971): Teratogenic effects of short-term and transitory zinc deficiency in rats. Teratology 4:199-204.

Hurley LS, Keen CL, Lonnerdal B (1980): Copper and fetal and neonatal development. In "Biological Roles of Copper. CIBA Foundation Symposium 79." New York: Excerpta Medica, pp 227-245.

Hurley LS, Shrader RE (1972): Congenital malformations of the nervous system in zinc-deficient rats. In Pfeiffer CC (ed): "Neurobiology of the Trace Metals Zinc and Copper." New York: Academic Press, pp 7-51.

Hurley LS, Swenerton H (1966): Congenital malformations resulting from zinc deficiency in rats. Proc Soc Exp Biol Med 123:692-696.

Hurley LS, Swenerton H (1971): Lack of mobilization of bone and liver zinc under teratogenic conditions of zinc deficiency in rats. J Nutr 101:597-604.

Hurley LS, Tao S (1972): Alleviation of teratogenic effects of zinc deficiency by simultaneous lack of calcium. Am J Physiol 222:322-325.

Hurley LS, Wooley DE, Rosenthal F, Timiras PS (1963): Influence of manganese on susceptibility of rats to convulsions. Am J Physiol 204:493-496.

Hurley LS, Wooley DE, Timiras PS (1961b): Threshold and pattern of electroshock seizures in ataxic manganese-deficient rats. Proc Soc Exp Biol Med 106:343-346.

Hurley LS, Wooten E, Everson GJ (1961c): Disproportionate growth in offspring of manganese-deficient rats. II. Skull, brain, and cerebrospinal fluid pressure. J Nutr 74:282-288.

Hurry VJ, Gibson RS (1982): The zinc, copper and manganese status of children with malabsorption syndromes and inborn errors of metabolism. Biol Trace Element Res 4:157-174.

Hyyppä M (1971): Hypothalamic monoamines and pineal dopamine during the sexual differentiation of the rat brain. Experientia 27:336-337.

Jacobson M (1978): "Developmental Neurobiology, 2nd ed." New York and London: Plenum.

Jameson S (1976): Effects of zinc deficiency in human reproduction. Acta Med Scand Suppl 593:5-89.

Joselow MM, Tobias E, Koehler R, Coleman S, Bogden J, Gause D (1978): Manganese pollution in the city environment and its relationship to traffic density. Am J Pub Health 68:557-560.

Katz S, Tenenhouse A (1973): The relation of adenyl cyclase to the activity of other ATP utilizing enzymes and phosphodieterase in preparations of rat brain; mechanism of stimulation of cyclic AMP accumulation by adrenalin, ovabain and Mn^{++}. Brit J Pharmacol 48:516-526.

Kawamoto JC, Halas ES (1984): Lasting morphological effects of mild perinatal zinc deficiency in the adult hippocampal formation. In: Frederickson CJ, Howell GA, Kasarskis EF (eds): " The Neurobiology of Zinc. Part B: Deficiency, Toxicity, and Pathology." New York: Alan R. Liss, Inc., pp 33-48.

Keen CL, Clegg MS, Lonnerdal B, Hurley LS (1983a): Whole blood manganese as an indicator of body manganese status. New Engl J Med 308:1230.

Keen CL, Hurley LS (1976): Copper supplementation in quaking mutant mice: reduced tremors and increased brain copper. Science 193:244-246.

Keen CL, Lonnerdal B, Hurley LS (1985): Manganese. In "Biochemistry of the Elements." New York: Plenum Publishing Co., In press.

Keen CL, Mark-Savage P, Lonnerdal B, Hurley LS (1983b): Teratogenic effects of D-penicillamine in rats: relation to copper deficiency. Drug-Nutr Interact 2:17-34.

Keinholz EW, Turk DE, Sunde ML, Hoekstra WG (1961): Effects of zinc deficiency in the diets of hens. J Nutr 75:211-221.

Klevay LM (1982): An appraisal of current human copper nutriture. In: Sorenson RJ (ed): "Inflammatory Diseases and Copper." Clifton, New Jersey: Humana Press, pp 123-136.

Lahey ME, Gubber GJ, Chase MS, Cartwright GE, Wintrobe MM (1952): Studies on copper metabolism. II. Hematologic manifestations of copper deficiency in swine. Blood 7:1053-1074.

Leach RM, Lilburn MS (1978): Manganese metabolism and its function. World Rev Nutr Diet 32:123-134.

Leach RM, Muenster AM (1962): Studies on the role of manganese in bone formation. I. Effect upon the mucopolysaccharide content of chick bone. J Nutr 78:51-56.

LeBaron F (1970): Metabolism of myelin constituents. In Lajtha A (ed): "Handbook of Neurochemistry, Vol 3, Metabolic Reactions in the Nervous System." New York and London: Plenum Press, pp 561-574.

Lehmann BH, Hansen JDL, Warren PG (1971): The distribution of copper, zinc and manganese in various regions of the brain and other tissues of children with protein-calorie malnutrition. Br J Nutr 26:197-202.

Levi-Montalcini R (1964): Events in the developing nervous system. In Purpura DP, Schade JP (eds): "Growth and Maturation of the Brain. Progress in Brain Research, Vol 4." Amsterdam: Elsevier, pp 1-29.

Lokken PM, Halas ES, Sandstead HH (1973): Influence of zinc deficiency on behavior. Proc Soc Exp Biol Med 144:680-682.

Lumsden CE (1950): Cyanide leucoencephalopathy in rats and observations on the vascular and ferment hypotheses of demyelinating diseases. J Neurol Neurosurg Psychiat 13:1-15.

Lund RD (1978): "Development and Plasticity of the Brain: An Introduction." New York: Oxford University Press.

Lyon MF (1951): Hereditary absence of otoliths in the house mouse. J Phsyiol London 114:410-418.

Lyon MF (1953): Absence of otoliths in the mouse: an effect of the pallid mutant. J Genet 51:638-650.

Lyons M, Insko WM (1937): Chondrodystrophy in the chick embryo produced by manganese deficiency in the diet of the hen. Ky Agric Exp Stn Bull No. 371.

Mandel P, Rein H, Harth-Edel S, Mardell R (1964): Distribution and metabolism of ribonucleic acid in the vertebrate central nervous system. In Richter D (ed): "Comparative Neurochemistry." London: Pergammon Press, pp 153-169.

Masters DG, Keen CL, Lonnerdal B, Hurley LS (1983): Zinc deficiency teratogenicity: The protective role of maternal tissue catabolism. J Nutr 113:905-912.

Mazus B, Falchuk KH, Vallee BL (1984): Histone formation, gene expression, and zinc deficiency in Euglena gracilis. Biochemistry 23:42-47.

McKenzie JM, Fosmire GJ, Sandstead HH (1975): Zinc deficiency during the latter third of pregnancy: Effects on fetal rat brain, liver, and placenta. J Nutr 105:1466-1475.

Menkes JH, Alter M, Steigleder GK, Weakley DR, Sung JH (1962): A sex-linked recessive disorder with retardation of growth, peculiar hair and focal cerebellar degeneration. Pediatrics 29:764-779.

Mills CF, Quarterman J, Chester JK, Williams RB, Dalgarno AC (1969): Metabolic role of zinc. Am J Clin Nutr 22:1240-1249.

Mills CF, Williams RB (1962): Copper concentrations and cytochrome-oxidase and ribonuclease activities in the brains of copper deficient lambs. Biochem J 85:629-632.

Moynahan EJ, Barnes PM (1973): Zinc deficiency and a synthetic diet for lactose intolerance. Lancet i:676-677.

Morgan RF, O'Dell BL (1977): Effect of copper deficiency on the concentrations of catecholamines and related enzyme activities in the rat brain. J Neurochem 28:207-213.

Morton MS, Elwood PC, Abernethy M (1976): Trace elements in water and congenital malformations of the central nervous system in South Wales. Br J Prev Soc Med 30:36-39.

Mutch PB, Hurley LS (1974): Effect of zinc deficiency during lactation on postnatal growth growth and development of rats. J Nutr 104:828-842.

Nystrom SHM (1965): Cytological aspects of the pathogenesis of intercranial aneurysms. In Fields WS, Sahs AL (eds): "Intercranial Aneurysms and Subarachnoid Hemorrhage." Springfield, IL: Thomas, pp 40-69.

Nystrom SHM (1968): Studies on the effect of hypocupremia on the rabbit circulatory system, with special reference to the vessels of the neck and base of the brain. Acta Neuropathol 10:209-217.

Oberleas D, Cladwell DJ, Prasad AS (1972): Trace elements and behavior: zinc. In Pfeiffer CC (ed): "Neurobiology of the Trace Elements Zinc and Copper." New York: Academic Press, pp 92-103.

O'Dell BL (1974): Role of zinc in protein synthesis. In Pories WJ, Strain WH, Hsu JM, Woosley RL (eds): "Clinical Applications of Zinc Metabolism." Springfield, IL: Thomas, pp 5-8.

O'Dell BL (1981): Roles for iron and copper in connective tissue biosynthesis. Phil Trans R Soc London B 294:91-104.

O'Dell BL, Kilburn KH, McKenzie WN, Thurston RJ (1978): The lung of the copper-deficient rat. Am J Pathol 91:413-432.

O'Dell BL, Prohaska JR (1983): Biochemical aspects of copper deficiency in the nervous system. In Dreosti IE, Smith RM (eds): "Neurobiology of the Trace Elements. Vol 1: Trace Element Neurobiology and Deficiencies." Clifton, NJ: Humana Press, pp 41-81.

O'Dell BL, Hardwick BC, Reynolds G (1961): Mineral deficiencies of milk and congenital malformations in rats. J Nutr 73:151-157.

O'Dell BL, Smith RM, King RA (1976): Effect of copper status on brain neurotransmitter metabolism in the lamb. J Neurochem 26:451-455.

Opsahl W, Abbott U, Kenney C, Rucker R (1984): Scoliosis in chickens: Responsiveness of severity and incidence to dietary copper. Science 225:440-442.

Papavasiliou PS, Kutt H, Miller ST, Rosal V, Wang YY, Aronson RB (1979): Seizure disorders and trace metals: Manganese tissue levels in treated epileptics. Neurology 29:1466-1473.

Prasad AS, Miale A, Farid Z, Schulent A, Sandstead HH (1963): Zinc metabolism in patients with the syndrome of iron deficiency anemia, hypogonadism and dwarfism. J Lab Clin Med 61:537-549.

Prasad AS, Oberleas D (1974): Thymidine kinase activity and incorporation of thymidine into DNA in zinc deficient tissue. J Lab Clin Med 83:634-639.

Prohaska JR, Wells WW (1975): Copper deficiency in the developing rat brain: evidence for abnormal mitochondria. J Neurochem 25:221-228.

Prohaska JR, Smith TL (1982): Effect of dietary or genetic copper deficiency on brain catecholamines, trace metals and enzymes in mice and rats. J Nutr 112:1706-1717.

Record IR, Dreosti IE (1979): Effects of zinc deficiency on the liver and brain thymidine kinase activity in the fetal rat. Nutr Rep Int 20:749-755.

Reinis S, Goldman JM (1980): "The Development of the Brain: Biological and Functional Perspectives." Springfield, IL: Charles C. Thomas.

Robinson LK, Hurley LS (1981a): Effect of maternal zinc deficiency or food restriction on rat fetal pancreas. 1. Procarboxylpeptidase A and Chymotrysinogen. J Nutr 111:858-868.

Robinson LK, Hurley LS (1981b): Effect of maternal zinc deficiency or food restriction on rat fetal pancreas. 2. Insulin and glucagon. J Nutr 111:869-877.

Rogers JM, Hurley LS (1984): Effects of maternal zinc deficiency on development of the fetal rat retina. Teratology 29:55A.

Rogers JM, Keen CL, Hurley LS (1984): Maternal zinc nutriture during pregnancy and lactation in the rat: Survivability and growth of offspring. Fed Proc 43:1052.

Rogers JM, Keen CL, Hurley LS (1985): Zinc deficiency in the pregnant Long Evans rat: Teratogenicity and tissue trace elements. Teratology, In press.

Rosso P, Hormazabal J, Winick M (1970): Changes in brain weight, cholesterol, phospholipid and DNA content in marasmic children. Amer J Clin Nutr 23:1275-1279.

Sandstead HH, Logan GW, Jacob RA, Strobel DA, Marks EO (1976): Zinc deficiency in pregnant Rhesus monkeys. Clin Res 24:504A.

Saxen L (1980): Neural induction: past, present, and future. In Hunt RK (ed): "Neural Development. Part I: Current Topics in Developmental Biology. Vol 15." New York: Academic Press, pp 409-418.

Schulz LA, Behrens H (1960): Beitrag zur pathogenese der kupfermangelencephalopathie des schflammes (swayback). Beitr Path Anat 122:282-288.

Sever LE, Emanuel I (1973): Is there a connection between maternal zinc deficiency and congenital malformations of the central nervous system in man. Teratology 7:117-119.

Shills ME, McCollom EV (1943): Further studies on the symptoms of manganese deficiency in the rat and mouse. J Nutr 26:1-31.

Shrader RE, Erway L, Hurley LS (1973): Mucopolysaccharide synthesis in the developing inner ear of manganese-deficient and pallid mutant mice. Teratology 8:257-266.

Smith H (1967): The distribution of antimony, arsenic, copper and zinc in human tissue. J Forens Sci Soc 7:97-102.

Smith JC, McDaniel EG, Farr FF, Halsted JA (1973): Zinc: a trace element essential in vitamin A metabolism. Science 181:954-955.

Smith RM (1981): Menkes disease and the role of copper in brain development. In Hetzel BS, Smith RM (eds): "Fetal Brain Disorders - Recent Approaches to the Problems of Mental Deficiency." Amsterdam: Elsevier-North Holland Biomedical Press, pp 365-387.

Smith RM (1983): Copper in the developing brain. In Dreosti IE, Smith RM (eds): "Neurobiology of the Trace Elements. Vol 1: Trace Element Neurobiology and Deficiencies." Clifton, NJ: Humana Press, pp 1-40.

Smith RM, Fraser FJ, Russell GR, Robertson JS (1977): Enzootic ataxia in lambs: Appearance of lesions in the spinal cord during fetal development. J Comp Path 87: 119-127.

Spais A, Palsson PA, Van Bogaert L (1961): Pathology of enzootic ataxia in lambs. Acta Neuropath (Berlin) 1:56-72.

Sperry WM (1962): The biochemistry of the brain during early development. In Elliott KAC, Page IH, Quastel JH (eds): "Neurochemistry." Springfield, IL: Charles C. Thomas, pp 55-84.

Spreen O, Tupper D, Risser A, Tuokko H, Edgell D (1984): "Human Developmental Neuropsychology." New York and Oxford: Oxford University Press.

Stankiewicz AJ, Falchuk KH, Vallee BL (1983): Composition and structure of zinc-deficient Euglena gracilis chromatin. Biochemistry 22:5151-515.

Stewart C, Katchan B, Collipp PJ, Clejan S, Pudalov S, Chen SY (1981): Zinc and birth defects. Pediat Res 15:515.

Sullivan JF, Heaney RO (1970): Zinc metabolism in alcoholic liver disease. Am J Clin Nutr 23:170-177.

Tagliamonte A, Tagliamonte P, Gessa GL (1970): Reserpine-like action of chloropromazine on rabbit basal ganglia. J Neurochem 17:733-738.

Tanaka Y (1977): Manganese: its neurological and teratological significance in man. Natl Mtg Chem Soc, Chicago.

Teague HS, Carpenter LE (1951): The demonstration of a copper deficiency in young guinea pigs. J Nutr 43:389-399.

Terr Harr GL, Griffing ME, Brandt M, Oberding DG, Kapron M (1975): Methyl-cyclopent-adienyl manganese tricarbonyl as an antiknock: composition and fate of manganese exhaust products. J Air Pollution Cont Assoc 25:858-860.

Tingey AH (1956): Human brain lipids at various ages in relation to myelination. J Ment Sci, London 102:851-855.

Turk DE, Sunde ML, Hoekstra WG (1959): Zinc deficiency experiments with poultry. Poultry Sci 38:1256.

Vallee BL (1976): Zinc biochemistry in the normal and neoplastic growth processes. In Shultz J, Ahmad F (eds): "Cancer Enzymology." New York: Academic Press, pp 159-195.

Verburg DL, Burd LI, Hoxtell EO (1974): Acrodermatitis enteropathica and pregnancy. Obstet Gynecol 44:233.

Vojnick C, Hurley LS (1977): Abnormal prenatal lung development resulting from maternal zinc deficiency in rats. J Nutr 107:862-872.

Wald N, Hambidge M (1977): Maternal serum-copper concentration and neural-tube defects. Lancet ii:560.

Walshe JM (1956): Wilson's disease: New oral therapy. Lancet 1:25-26.

Warkany J, Petering HG (1972): Congenital malformations of the central nervous system in rats produces by maternal zinc deficiency. Teratology 5:319-344.

Warkany J, Petering HG (1973): Congenital malformations of the brain produced by short-term zinc deficiency in rats. Am J Ment Defic 77:645-653.

Warren PJ, Earl CJ, Thompson RHS (1960): The distribution of copper in human brain. Brain 83:709-717.

Wedler FC, Denmen RB, Roby WG (1982): Glutamine synthetase from ovine brain is a manganese (11) enzyme. Biochemistry 24:6389-6396.

Wiley MJ, Joneja MG (1978): Neural tube lesions in the offspring of hamsters given single oral doses of lathyrogens early in gestation. Acta Anat 100:347-353.

Winick M (1968): Changes in nucleic acid and protein content of the human brain during growth. Pediatr Res 2:352-355.

Winick M (1976): "Malnutrition and Brain Development." London: Oxford University Press.

Winick M, Rosso P, Waterlow J (1970): Cellular growth of cerebrum, cerebellum, and brain stem in normal and marasmic children. Exptl Neurol 26:393-400.

Yamada T, Otomo S, Tanaka Y, Sasajmia M, Ohzehi M (1979): Reproductive studies of D-penicillamine in rats. (2) Teratogenicity study. Pharmacometrics 18:561-569.

Yip GB, Dain JA (1970): The enzymic synthesis of ganglioside. II. UDP-galactose:N-acetylgalactosaminyl-(CN-acetylneuraminyl) galactosyl-glucosyl-ceramide galactosyltransferase in rat brain. Biochem Biophys Acta 206:252-260.

Zidenberg-Cherr S, Keen CL, Hurley LS (1985): The effects of manganese deficiency during prenatal and postnatal development on mitochondrial structure and function. Biol Trace Element Res, In press.

Zidenberg-Cherr S, Keen CL, Lonnerdal B, Hurley LS (1983): Superoxide dismutase activity and liquid peroxidation: developmental correlations affected by manganese deficiency. J Nutr 113:2498-2504.

Zimmerman AW, Matthieu JM, Quarles RH, Brady RO, Hsu JM (1976): Hypomyelination in copper deficient rats. Arch Neurol 33:111-119.